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(54) **ELECTRICAL FIELD STIMULATION OF EUKARYOTIC CELLS**

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(58) **Field of Classification Search** ..... None
See application file for complete search history.

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(57) **ABSTRACT**

Methods of identifying activators and inhibitors of voltage-gated ion channels are provided in which the methods employ electrical field stimulation of the cells in order to manipulate the open/close state transition of the voltage-gated ion channels. This allows for more convenient, more precise experimental manipulation of these transitions, and, coupled with efficient methods of detecting the result of ion flux through the channels, provides methods that are especially suitable for high throughput screening.

7 Claims, 57 Drawing Sheets

ELECTRICAL FIELD STIMULATION OF EUKARYOTIC CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

The subject application is related to co-pending provisional application No. 60/304,955, filed Jul. 12, 2001, to which priority is claimed under 35 USC § 119(e).

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not applicable.

REFERENCE TO MICROFICHE APPENDIX

Not applicable.

FIELD OF THE INVENTION

The present invention is directed to methods and associated apparatuses for stimulating eukaryotic cells by the application of electric fields. The electric fields are produced by certain arrangements of electrodes that create an electric potential difference in the environment of the cells, resulting in a change in membrane potential of the cells. The change in membrane potential affects various physiological processes within the cells, including the opening and closing of voltage-gated ion channels. The ability to alter the open/close transitions of voltage-gated ion channels by the application of electric fields as described herein provides for novel methods of screening compounds for the ability to modulate the activity of voltage-gated ion channels.

BACKGROUND OF THE INVENTION

Certain molecular events in eukaryotic cells depend on the existence or magnitude of an electric potential gradient across the plasma (i.e., outer) membrane of the cells. Among the more important of such events is the movement of ions across the plasma membrane through voltage-gated ion channels. Voltage-gated ion channels form transmembrane pores that open in response to changes in cell membrane potential and allow ions to pass through the membrane. Voltage-gated ion channels have many physiological roles. They have been shown to be involved in maintaining cell membrane potentials and controlling the repolarization of action potentials in many types of cells (Bennett et al., 1993, Cardiovascular Drugs & Therapy 7:195-202; Johnson et al., 1999, J. Gen. Physiol. 113:565-580; Bennett & Shin, "Biophysics of voltage-gated sodium channels," in *Cardiac Electrophysiology: From Cell to Bedside*, 3rd edition, D. Zipes & J. Jalife, eds., 2000, W. B. Saunders Co., pp. 67-86; Bennett & Johnson, "Molecular physiology of cardiac ion channels," Chapter 2 in *Basic Cardiac Electrophysiology and Pharmacology*, 1st edition, A. Zasa & M. Rosen, eds., 2000, Harwood Academic Press, pp. 29-57). Moreover, mutations in sodium, calcium, or potassium voltage-gated ion channel genes leading to defective channel proteins have been implicated in a variety of disorders including the congenital long QT syndromes, ataxia, migraine, muscle paralysis, deafness, seizures, and cardiac conduction diseases, to name a few (Bennett et al., 1995, Nature 376:683-685; Roden et al., 1995, J. Cardiovasc. Electrophysiol. 6:1023-1031; Kors et al., 1999, Curr. Opin. Neurol. 12:249-254; Lehmann et al., 1999, Physiol. Rev. 79:1317-1372; Holbauer & Heufelder, 1997, Eur. J. Endocrinol. 136:588-589; Naccarelli & Antzelevitch, 2000, Am. J. Med. 110:573-581).

Several types of voltage-gated ion channels exist. Voltage-gated potassium channels establish the resting membrane potential and modulate the frequency and duration of action potentials in neurons, muscle cells, and secretory cells. Following depolarization of the membrane potential, voltage-gated potassium channels open, allowing potassium efflux and thus membrane repolarization. This behavior has made voltage-gated potassium channels important targets for drug discovery in connection with a variety of diseases. Dysfunctional voltage-gated potassium channels have been implicated in a number of diseases and disorders. Wang et al., 1998, Science 282: 1890-1893 have shown that the voltage-gated potassium channels KCNQ2 and KCNQ3 form a heteromeric potassium ion channel known as the "M-channel." Mutations in KCNQ2 and KCNQ3 in the M-channel are responsible for causing epilepsy (Biervert et al., 1998, Science 279:403-406; Singh et al., 1998, Nature Genet. 18:25-29; Schroeder et al., Nature 1998, 396:687-690).

Voltage-gated sodium channels are transmembrane proteins that are essential for the generation of action potentials in excitable cells (Catterall, 1993, Trends Neurosci. 16:500-506). In mammals, voltage-gated sodium channels consist of a macromolecular assembly of α and β subunits with the α subunit being the pore-forming component. α subunits are encoded by a large family of related genes, with some α subunits being present in the central nervous system (Noda et al., 1986, Nature 322:826-828; Auld et al., 1988, Neuron 1:449-461; Kayano et al., 1988, FEBS Lett. 228:187-194) and others in muscle (Rogart et al., 1989, Proc. Natl. Acad. Sci. USA 86:8170-8174; Trimmer et al., 1989, Neuron 3:33-49).

Voltage-gated calcium channels are transmembrane proteins that in the open configuration allow the passive flux of Ca^{2+} ions across the plasma membrane, down the electrochemical gradient. They mediate various cell functions, including excitation-contraction coupling, signal transduction, and neurotransmitter release.

Current methods of drug discovery often involve assessing the biological activity (i.e., screening) of tens or hundreds of thousands of compounds in order to identify a small number of those compounds having a desired activity. In many high throughput screening programs, it is desirable to test as many as 50,000 to 100,000 compounds per day. Unfortunately, current methods of assaying the activity of voltage-gated ion channels are ill suited to the needs of a high throughput screening program. Current methods often rely on electrophysiological techniques. Standard electrophysiological techniques involve "patching" or sealing against the cell membrane with a glass pipette followed by suction on the glass pipette, leading to rupture of the membrane patch (Hamill et al., 1981, Pflugers Arch. 391: 85-100). This has limitations and disadvantages. Accessing the cell interior may alter the cell's response properties. The high precision optical apparatuses necessary for micromanipulating the cells and the pipettes make simultaneous recording from more than a few cells at a time impossible. Given these difficulties, the throughput that can be achieved with electrophysiological techniques falls far short of that necessary for high throughput screening.

Various techniques have been developed as alternatives to standard methods of electrophysiology. For example, radioactive flux assays have been used in which cells are loaded with a radioactive tracer (e.g., $^{86}Rb^{+}$, $^{22}Na^{+}$, [^{14}C]-guani-

dinium) and the efflux of the dye is monitored. Cells loaded with the tracer are exposed to compounds and those compounds that either enhance or diminish the efflux of the tracer are identified as possible activators or inhibitors of ion channels in the cells' membranes.

Assays that measure the change in a cell's membrane potential due to the change in activity of an ion channel have been developed. Such assays often employ voltage sensitive dyes that redistribute between the extracellular environment and the cell's interior based upon a change in membrane potential and that have a different fluorescence spectrum depending on whether they are inside or outside the cell. A related assay method uses a pair of fluorescent dyes capable of fluorescence resonance energy transfer to sense changes in membrane potential. For a description of this technique, see González & Tsien, 1997, Chemistry & Biology 4:269-277. See also González & Tsien, 1995, Biophys. J. 69:1272-1280 and U.S. Pat. No. 5,661,035. Other methods employ ion selective indicators such as calcium dependent fluorescent dyes to monitor changes in Ca^{2+} influx during opening and closing of calcium channels.

Ideally, methods of screening against voltage-gated ion channels require that the transmembrane potential of the cells being assayed be controlled and/or that the ion channels studied be cycled between open and closed states. This has been done in various ways. In standard electrophysiological techniques, the experimental set-up allows for direct manipulation of membrane potential by the voltage clamp method (Hodgkin & Huxley, 1952, J. Physiol. (Lond.) 153:449-544), e.g., changing the applied voltage or injecting various ions into the cell. In other methods, changing the extracellular K^+ concentration from a low value (e.g., 5 mM) to a higher value (e.g., 70-80 mM) results in a change in the electrochemical potential for K^+ due to the change in the relative proportion of intracellular and extracellular potassium. This results in a change in the transmembrane electrical potential towards a more depolarized state. This depolarization can activate many voltage-gated ion channels, e.g., voltage-gated calcium, sodium, or potassium channels. Alternatively, Na^+ channels can be induced into an open conformation by the use of toxins such as veratridine or scorpion venom (Strichartz et al., 1987, Ann. Rev. Neurosci. 10:237-267; Narahashi & Harman, 1992, Meth. Enzymol. 207:620-643). While sometimes effective, such experimental manipulations may alter the channel pharmacology, can be awkward to perform, and can lead to artifactual disturbances in the system being studied.

Electrical field stimulation of cells has been performed on a single cell by sealing a glass microelectrode to the cell membrane. Rupture of the sealed patch of cell membrane resulted in an electrical connection between the interior fluid in the glass microelectrode and the fluid within the cell that was used to stimulate the cell via an electronic pulse generator. The electrophysiological response of the cell was measured via a sensitive electronic amplifier. The disadvantage of this technique is that only one cell at a time was tested and it is a tedious and time consuming operation to seal the microelectrode to an individual cell.

HEK293 cells have been grown on a silicon chip made up of an array of field-effect transistors. Some of the cells were positioned over the gate region of the transistors, thus having portions of their plasma membranes overlying the source and the drain. When a patch pipette in such cells manipulated the intracelluiar voltage, Maxi-K potassium channels in the cells' plasma membranes were opened. This led to current flow in the region between the cells' membrane and the transistor. This current flow modulated the source-drain current, which could be detected by an appropriate device. The chip plus cells was said to have potential as a sensor and as a prototype for neuroprosthetic devices. See Straub et al., 2001, Nature Biotechnol. 19:121-124; Neher, 2001, Nature Biotechnol. 19:114.

SUMMARY OF THE INVENTION

The present invention is directed to methods of identifying activators and inhibitors of voltage-gated ion channels in which the methods employ electrical field stimulation of the cells via extracellular electrodes in order to manipulate the open/close state transitions of the voltage-gated ion channels. This allows for more convenient, more precise manipulation of these transitions, and, coupled with efficient methods of detecting ion flux or membrane potential, results in methods that are especially suitable for high throughput screening in order to identify substances that are activators or inhibitors of voltage-gated ion channels.

The present invention also provides apparatuses for use in the above-described methods. In particular, modifications of standard multiwell tissue culture plates are provided where the modified multiwell tissue culture plates have electrodes that can alter the transmembrane electric potential of cells in the wells of the plates, thus altering the ratio of open/close states of voltage-gated ion channels in the cells.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1A** shows a top view of one embodiment of the present invention. This embodiment comprises a glass slide **1** in which or upon which are a gold positive electrode **2** and a gold negative electrode **3** spaced such that a gap **4** of about 25 μm to 100 μm exists between the electrodes. The electrodes together with spacers **5** (here shown as plastic strips) arranged generally at right angles to the electrodes define a series of wells **6** about 100 μm deep into which cells can be placed and/or grown. FIG. **1B** shows a cross-sectional side view of the embodiment of FIG. **1A**. In this embodiment, the identities of the positive and negative electrodes can be interchanged, if desired. The electrodes need not be made from gold; other conductive materials may be used. Also, the spacers need not be plastic; other non-conductive materials may be used.

FIG. **2A** shows a top view of an embodiment of the present invention in which a typical 96 well plate contains electrodes within each well. FIG. **2B** shows a cross-sectional side view of one of the wells in FIG. **2A**. The well has a first electrode **1** (here shown as a positive electrode) on the side **2** of the well, a second electrode **3** (here shown as a negative electrode) on the bottom **4** of the well, a strip of an optional insulating material **5** on the bottom of the well, and a cell **6** at the bottom of the well. A single cell is shown merely for convenience of illustration; in most cases a plurality of cells would be in the bottom of the well. The sides **2** of the well are made of a non-conducting material such as plastic and the bottom of the well is made from a conducting material such as indium tin oxide (ITO). The well is shown with a fluid level **7** sufficient to completely cover the cell **6** and the second electrode **3** at the bottom **4** of the well and to reach the first electrode **1** on the side **2** of the well. The well is not drawn to scale with respect to FIG. **2A**. FIG. **2C** shows an alternative arrangement of electrodes in a well. In this embodiment, both the positive electrode **1** and the negative electrode **2** are in the bottom **3** of the well. In this embodiment, the sides **4** and bottom **3** of the well are made of

the amount of current required to initially charge the plates with only a miniscule current required to maintain the charge between the plates. An external electric field is generated that can be used to depolarize the cells. The external electric field density is reduced by a high dielectric between the plates as is used with an authentic capacitor and is maximal with a low dielectric such as teflon or mylar or no dielectric. The external field density is further enhanced by placing the plates very close together, but the optimal separation may be determined empirically.

FIG. **36**B shows an embodiment comprising a concurrent lead design. The concurrent lead comprises an internal wire **3655** and an external wire **3650**. The internal wire passes through the top plate **3610** and dielectric plate **3620** and is attached or integral to the bottom plate **3630**. The external wire is attached or integral to the top plate **3610**. Those skilled in the art will recognize that the foregoing arrangement of the leads may be reversed. FIG. **36**C shows an embodiment comprising edge leads **3660** and **3665**. Edge lead **3660** is attached or integral to top plate **3610** and edge lead **3665** is attached or integral to bottom plate **3630**.

Some of the embodiments of the subject invention include the following:

A method of characterizing the biological activity of a candidate compound comprising.

exposing one or more cells to said compound; repetitively exposing said one or more cells to one or more electric fields so as to effect a controlled change in transmembrane potential of said one or more cells; and monitoring, without using a patch clamp, changes in the transmembrane potential of said one or more cells.

The above method, where the monitoring comprises detecting fluorescence emission from an area of observation containing said one or more cells.

The above method, where the electric fields are biphasic.

The above method, additionally comprising limiting spatial variation in electric field intensity so as to minimize irreversible cell electroporation.

The above method, where one or more electrical fields may cause an ion channel of interest to cycle between different voltage dependent states.

The above method, where the one or more electrical fields cause an ion channel of interest to open.

The above method, where the one or more electrical fields cause an ion channel of interest to be released from inactivation.

The above method, where the one or more cells comprise a voltage sensor selected from the group consisting of a FRET based voltage sensor, an electrochromic transmembrane potential dye, a transmembrane potential redistribution dye, an ion sensitive fluorescent or luminescent molecule and a radioactive ion.

The above method, where the one or more cells comprise a voltage regulated ion channel.

The above method, where the voltage regulated ion channel is selected from the group consisting of a potassium channel, a calcium channel, a chloride channel and a sodium channel.

The above method, where the electric field exhibits limited spatial variation in intensity in the area of observation of less than about 25% from. a mean intensity in that area.

The above method, where the one or more electrical fields varies over an area of observation by no more than about 15% from the mean electrical field at any one time.

The above method, where the one or more electrical fields varies over an area of observation by no more than about 5% from the mean electrical field at any one time.

The above method, where the one or more electrical fields comprises stimulation with either a square wave-form, a sinusoidal wave-form or a saw tooth wave-form.

The above method, where the one or more electrical fields have an amplitude within the range of about 10 V/cm to about 100 V/cm.

The above method, where the one or more electrical fields have an amplitude within the range of about 20 V/cm to about 80 V/cm.

The above method, where the one or more electrical fields are repeated at a frequency of stimulation that is greater than or equal to the reciprocal of the transmembrane time constant of said one or more cells.

The above method, where the one or more electrical fields are repeated at a frequency of stimulation within the range of zero to 1 kHz.

The above method, where the one or more electrical fields have a pulse duration within the range of about 100 microseconds to about 20 milliseconds.

The above method, where the transmembrane potential is developed across the plasma membrane of said one or more cells.

A method of assaying the biochemical activity of a compound against a target ion channel comprising.

selecting a cell line having a normal resting transmembrane potential corresponding to a selected voltage dependent state of said target ion channel; expressing said target ion channel in a population of cells of said selected cell line; exposing said population of cells to said compound; repetitively exposing said population of cells to one or more electric fields so as to effect a controlled change in transmembrane potential of said one or more cells; and monitoring changes in the transmembrane potential of said one or more cells.

The above method, where the target ion channel is exogenously expressed in said cell line.

The above method, where the cell line is transfected with nucleic acid encoding said target ion channel.

The above method, where the cell line expresses insignificant levels of other ion channels.

The above method, where the cell line is selected from the group consisting of CUL,LTK(−), and CHO-M.

The above method, where the target ion channel is a sodium channel, and wherein said population of cells is selected from the group consisting of CHL cells, LTK(−) cells, and CHO-K1 cells.

The above method, where the target ion channel is a sodium channel, and wherein said population of cells is selected from the group consisting of HEK-293 cells, RBL cells, F11 cells, and HL5 cells.

The above method, where the target ion channel is a potassium channel, and wherein said population of cells is selected from the group consisting of CHL cells, LTK(−) cells, and CHO-K1 cells.

The above method, where the target ion channel is a calcium channel, and wherein said population of cells is selected from the group consisting of CHL cells, LTK(−) cells, and CHO-K1 cells.

A method of assaying ion channel activity comprising.

exposing at least one cell to a plurality of electric field pulses so as to create a controlled change in transmembrane potential and so as to activate an ion channel of interest; and detecting ion channel activity by detecting one or more changes in transmembrane potential without using a patch clamp.

The above method, where the at least one cell comprises a voltage sensor selected from the group consisting of a

FRET based voltage sensor, an electrochromic transmembrane potential dye, a transmembrane potential redistribution dye, an ion sensitive fluorescent or luminescent molecule and a radioactive ion.

The above method, where the voltage sensor comprises a FRET based voltage sensor.

The above method, where the ion channel of interest is a voltage regulated ion channel.

The above method, where the plurality of electric field pulses cause said ion channel of interest to cycle between different voltage dependent states.

The above method, where the at least one cell is an eukaryotic cell.

The above method, where the at least one cell is a non-excitable cell.

The above method, where the at least one cell is a prokaryotic cell.

The above method, where the at least one cell is a tissue culture cell.

The above method, where the at least one cell is a primary cell line.

The above method, where the at least one cell is part of an intact living organism.

A method of assaying ion channel activity comprising.

expressing a selected target ion channel in at least one cell; expressing a selected counter ion channel in said at least one cell; exposing said at least one cell to a plurality of electric field pulses so as to create a controlled change in transmembrane potential and so as to activate said counter ion channel; and monitoring the transmembrane potential of said at least one cell.

The above method, where a transmembrane potential change is detected when said ion channel of interest is blocked.

The above method, where the ion channel of interest comprises a ligand gated ion channel.

The above method, where the counter channel comprises a sodium channel.

A method of modifying the transmembrane potential of a cell comprising repetitively applying biphasic electric field pulses to said cell, wherein said pulses have a maximum amplitude of less than approximately 90 V/cm, wherein said pulses are applied at a rate of at least about 1 per second, and wherein the total duration of each pulse is at least about 1 millisecond.

The above method, where the maximum amplitude is approximately 20 to 40 V/cm.

The above method, where the pulse duration is approximately 2 to 10 milliseconds per phase.

The above method, where the pulses are applied at a rate of approximately 20 to 100 pulses per second.

A method of characterizing the biological activity of a candidate compound comprising.

placing one or more cells into an area of observation in a sample well; exposing said one or more cells to said compound; repetitively exposing said one or more cells to a series of biphasic electric fields at a rate of approximately 20 to 100 pulses per second, wherein said electric fields exhibit limited spatial variation in intensity in the area of observation of less than about 25% from a mean intensity in that area, and wherein said electric fields produce a controlled change in transmembrane potential of said one or more cells; and monitoring changes in the transmembrane potential of said one or more cells by detecting fluorescence emission of a FRET based voltage sensor from, an area of observation containing said one or more cells.

The above method, where the one or more electrical fields cause an ion channel of interest to open.

The above method, where the one or more electrical fields cause an ion channel of interest to be released from inactivation.

The above method, where the one or more cells comprise a voltage regulated ion channel.

The above method, where the voltage regulated ion channel is selected from the group consisting of a potassium channel, a calcium channel, a chloride channel and a sodium channel.

The above method, where the one or more electrical fields likely vary over an area of observation by no more than about 15% from the mean electrical field at any one time.

The above method, where the one or more electrical fields varies over an area of observation by no more than about 5% from the mean electrical field at any one time.

The above method, where the one or more electrical fields are selected from a square wave-form, a sinusoidal wave-form or a saw tooth wave-form.

A high throughput screening system comprising.

a plurality of wells having a high transmittance portion through which cells present in said wells are optically observable in an area of observation; two electrodes in each of said plurality of wells; an optical detector configured to detect light emanating from said wells through said high transmittance portion; a power supply connected to said electrodes; wherein said power supply and said electrodes are configured to apply a series of electric fields to cells within said area of observation, said electric fields having a spatial variation of less than about 25% of a mean field intensity within said area of observation, said electric fields being effective to controllably alter the transmembrane potential of a portion of said cells; a data processing unit configured to interpret said light emanating from said wells, through said high transmittance portion as ion channel activity resulting from said transmembrane potential alterations.

The above high throughput screening system, where the pluarality of wells are located in a multiwell plate.

The above high throughput screening system, where the high transmittance portion is made from a material selected from the group consisting of glass, quartz, cycloolefin, Aclar, polypropylene, polyethylene and polystyrene.

The above high throughput screening system, where the high transmittance portion exhibits less fluorescence when excited with UV light in the range of 250 nm to 400 nm than polystyrene.

The above high throughput screening system, where the electrodes are located in a well of said plurality of wells.

The above high throughput screening system, where the electrodes are located in a bottom layer of said plurality of wells.

The above high throughput screening system, where the multiwell plate comprises up to 96 wells.

The above high throughput screening system, where the multiwell plate comprises greater than 96 wells.

The above high throughput screening system, where the multiwell plate comprises greater than 384 wells.

The above high throughput screening system, where the electrodes are made of a material selected from the group consisting of gold, platinum, palladium, chromium, molybdenum, iridium, tungsten, tantalum and titanium.

The above high throughput screening system, where the multiwell plate comprises optically opaque materials or pigments to reduce the transmission of light.

The above high throughput screening system, where the electrodes are separated by a gap within the range of about 1 to 4 mm.

The above high throughput screening system, where the electrodes are separated by a gap within the range of about 0.1 to 1 mm.

1.0 The above high throughput screening system, where the electrodes are separated by a gap within the range of about 0.01 to 0.1 mm.

The above high throughput screening system, where the electrodes are charged to create an electrical field intensity of between 5 to 100 V/cm across said gap, and wherein the total charge transferred across the surface area of the electrically conductive material, in fluidic connection with the interior of the well is less than or equal to 100 μC/mm2.

The above high throughput screening system, where the plurality of wells further comprise an insulator orientated and configured so as to create an area of observation within said well in which. the electrical field intensity varies by no more than 10% from the mean electrical field intensity when said at least two strips of electrically conductive material are charged to create an electrical field intensity of between 5 to 100 V/cm across said gap, and. wherein the total charge transferred across the surface area of the electrically conductive material, in fluidic connection with the interior of the well is less than or equal to looptC/mm2.

The above high throughput screening system, where the plurality of wells further comprise at least two satellite electrical conductors.

A high throughput screening system comprising.

sample wells; liquid handling stations for adding reagents and/or cells to said sample wells; and means for controlling the transmembrane potential of cells in said sample wells so as to selectively cause ion channel activity.

means for optically monitoring changes in said transmembrane potential.

The above high throughput screening system, where the means comprises electrodes configured to create an electric field having a spatial variation of less than about 25% of a mean field intensity within an area of observation.

The above high throughput screening system, where the means for controlling the transmembrane potential comprise an electrode array assembly.

The above high throughput screening system, where the electrode assembly array comprises 8 electrode assemblies.

The above high throughput screening system, where the electrode assembly array comprises 96 electrode assemblies.

The above-high throughput screening system, where the electrode assembly array comprises greater than 96 electrode assemblies.

The above high throughput screening system, where the system further comprises means for retractably moving said electrode assembly into and out of the wells of a multiwell plate.

The above high throughput screening system, where the means for controlling the transmembrane potential comprises electrical conductors with two substantially parallel planar surfaces.

The above high throughput screening system, where the electrical conductors are separated by a gap within the range of 1 to 4 mm.

The above high throughput screening system, where the electrical conductors are separated 5 by a gap within the range of 0.1 to 1 mm.

The above high throughput: screening system, where the electrical conductors further comprise a first insulator.

The above high throughput screening system, where the first insulator comprises two planar surfaces orientated perpendicular to said substantially parallel planar surfaces of said electrical conductors and substantially parallel with respect to each other.

The above high throughput: screening system, where the electrical conductors further comprise a second insulator attached to said at least two electrical conductors, wherein said second insulator is interposed in said gap between said at least two electrical conductors to define the depth of said aqueous solution between said at least two electrical conductors.

The above high throughput: screening system, where the first insulator is composed of allow fluorescence material, wherein. said low fluorescence material exhibits less fluorescence when excited with UV light in the range 250 nm to 400 nm than polystyrene of comparable size.

The above high throughput screening system, where the second insulator is composed of a low fluorescence material, wherein said low fluorescence material exhibits less fluorescence when excited with UV light in the range 250 nm to 400 nm than polystyrene of comparable size.

The above high throughput screening system, where the first insulator comprises an insulator selected from the group consisting of plastic, glass and ceramic.

The above high throughput screening system, where the plastic is selected from the group consisting of nylon, polystyrene, Teflon (tetrafluoroethylene), polypropylene, polyethylene, poly-vinyl chloride, and cycloolefin.

The above high throughput screening system, where the electrical conductors comprise a conductor selected from the group consisting of gold, platinum, titanium, tungsten, molybdenum, iridium, vandium, Nb, Ta, stainless steel and graphite.

The above high throughput screening system, where the electrical conductors comprise a surface treatment to reduce electrolysis.

The above high throughput screening system, where the surface treatment to reduce electrolysis comprises platinum black, gold black, iridium/iridium oxide, titanium/titanium nitride or polypyrrole films.

The above high throughput screening system, where the electrical field intensity varies by no more than 10% from the mean electrical field intensity when said at least two electrical conductors are charged to create an electrical field intensity of between 5 to 100 V/cm across said gap, wherein the total charge transferred across the surface area of the electrical conductors in contact with said aqueous solution is less than or equal to 100 μC/mm2.

The above high throughput screening system, where the electrical field intensity varies by no more than 5% from the mean electrical field intensity when said at least two electrical conductors are charged to create an electrical field intensity of between 5 to 100 V/cm across said gap, wherein the total charge transferred across the surface area of the electrical conductors in contact with said aqueous solution is less than or equal to 100 μC/mm2.

A method of screening a plurality of drug candidate compounds against a target ion channel comprising.

expressing said target ion channel in a population of host cells; placing a plurality of said host cells into each of a plurality of sample wells; adding a candidate drug compound to at least: one of said plurality of sample wells; and modulating the transmembrane potential of host cells in said plurality of sample wells with a repetitive application of

electric fields so as to set said transmembrane potential to a level corresponding to a pre-selected voltage dependent state of said target ion channel.

The above method, additionally comprising selecting a host: cell line having a normal resting transmembrane potential corresponding to a second pre-selected voltage dependent state of said target ion channel.

The above method, where the electric fields are biphasic.

The above method, where electric fields cause an ion channel of interest to cycle between different voltage dependent states.

The above method, where the electric fields cause an ion channel of interest to open.

The above method, where the electric fields cause an ion channel of interest to be released from inactivation.

The above method, where the one or more cells comprise a voltage sensor selected from the group consisting of a FRET based voltage sensor, an electrochromic transmembrane potential dye, a transmembrane potential redistribution dye, an ion sensitive fluorescent or luminescent molecule and a radioactive ion.

The above method, where the target ion channel is selected from the group consisting of a potassium channel, a calcium channel, a chloride channel and a sodium channel.

The above method, where the one or more electrical fields comprises stimulation with either a square wave-form, a sinusoidal wave-form or a saw tooth wave-form.

The above method, where the one or more electrical fields have an amplitude within the range of about 10 V/cm to about 100 V/cm.

The above method, where the one or more electrical fields have an amplitude within the range of about 20 V/cm to, about 80 V/cm.

An assay plate and electrode assembly comprising at least one sample well having electrodes placed therein, wherein said electrodes are positioned with respect to the bottom surface of the well to provide an electric field adjacent to said bottom surface that varies by less than about 10% from a mean field intensity over at least about 20% of the surface area of said bottom surface.

The above assembly, where the electrodes comprise plate electrodes extending down into said well such that bottom ends of said electrodes are adjacent to but not in contact with said bottom surface.

The above assembly, comprising two electrodes per sample well. The above assembly, comprising more than two electrodes per sample well.

The above assembly, where the electrodes are plated onto said bottom surface of said well. The above assembly, where the bottom surface comprises a high optical transmittance portion.

The above assembly, where the high transmittance portion is made from a material selected from the group consisting of glass, quartz, cycloolefin, Aclar, polypropylene, polyethylene and polystyrene.

The above assembly, where the high transmittance portion exhibits less fluorescence when excited with UV light in the range of 250 nm to 400 nm than polystyrene.

The above assembly, where the electrodes are located in a wall of said plurality of wells.

The above assembly, where the plate comprises up to 96 wells.

The above assembly, where the plate comprises greater than 96 wells.

The above assembly, where the plate comprises greater than 384 wells.

The above assembly, where the electrodes are made of a material selected from the group consisting of gold, platinum, palladium, chromium, molybdenum, iridium, tungsten, tantalum and titanium.

The above assembly, where the electrodes are separated by a gap within the range of about 1 to 4 mm.

The above assembly, where the electrodes are separated by a gap within the range of about 0.1 to 1 mm.

The above assembly, where the electrodes are separated by a gap within the range of about 0.01 to 0.1 mm.

A bottom panel for a multi-well plate comprising.

at least one row of high transmittance regions with positions corresponding to well locations; a first: strip of conductive material extending along said row and overlapping a first portion of said well locations; and a second strip of conductive material extending along said row and overlapping a second portion of said well locations.

The above bottom panel, additionally comprising a first: electrical contact proximate to an end of said first strip and a second electrical contact proximate to an end of said second strip.

An assay apparatus comprising.

a sample well; a first pair of electrodes positioned within said sample well; at least one additional satellite electrode positioned within said sample well.

The above assay apparatus, where the at least one additional satellite electrode comprises second and third pairs of electrodes.

The above assay apparatus, where the satellite electrodes are charged to a potential less than that of the first pair of electrodes.

The above assay apparatus, where the electrodes are positioned with respect to the bottom surface of the well to provide an electric field adjacent to said bottom surface that varies by less than about 10% from a mean field intensity over at least about 20% of the surface area of said bottom surface.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties. Furthermore, for general information, PCT Publication No. PCT/US01/21652 is incorporated herein in its entirety to the extent it is accurate and not inconsistent with the teachings herein. All patents, patent applications, publications, texts and references discussed or cited herein are understood to be incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually set forth in its entirety. In addition, all references, patents, applications, and other documents cited in an Invention Disclosure Statement, Examiner's Summary of Cited References, or otherwise entered into the file history of this application are taken to be incorporated by reference into this specification for the benefit of later applications claiming priority to this application. Finally, all terms not specifically defined are first taken to have the meaning given through usage in this disclosure, and if no such meaning is inferable, their normal meaning.